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Original Article

PRODUCTION OF SINGLE CELL OIL BY LOCAL ISOLATE OF *MUCOR* SPECIES USING BY-PRODUCTS AS CARBON AND NITROGEN SOURCES AND DETERMINATION OF FATTY ACIDS PROFILE

RAJI. T. N. AL-ZAMILY[1], AMAAL. K. G. AL-ASSADI[2] & MOHAMMED. A. S. ISSA[3]

[1]*College of Agriculture, University of Sumer, Iraq*

[2]*Department of Food Science and Bio- Technologies, College of Agriculture, University of Basra, Iraq*

[3]*Department of Biology, College of Sciences, University of Dhi Qar, Iraq*

ABSTRACT

Eight local fungi isolates from different resources, namely soil, some agricultural by products, vegetable, dairy and meat products were isolated, identified and belonged to Mucor species (sp.). A primary screening was conducted to determine the capacity of producing of single cell oil at rate exceeding 20% of dried biomass. Seven isolates were selected for oil producing without toxicity when examined via bioassay with Artemia salina. Also, a secondary screening was conducted to evaluate the quality of the obtained oil from the seven isolates. The isolate Mucor sp.(Mu3) was found to produce high oil content and valued 5.474gm/L from 10.963gm/L of dry biomass with 49.93% total lipid.The best and the highest rate of polyunsaturated fatty acids (PUFA) profile was ϒ-Linolenic acid, Eicosapentaenoic acid, Docosapentaenoic acid (DPA), Docosahexaenoic acid at 10.95%, 4.6%, 0.39%, 1.47% respectively. The optimum isolate was studied for best carbon and nitrogen sources to support fungal growth and oil productivity. It was found that rice bran and chicken feather were the best carbon and nitrogen sources at rates of 7.5% gm/ml and 0.1% gm/ml respectively in liquid culture at 6-7pH, 10^6 or 10^7 spores/ml inoculums volume in shaking incubator at 28±1 ^oC for 7 days at a speed of 200 rounds per minute. There was significant differences at ($p < 0.01$) in the productivity for some sources used in this study that leading to conclude the possibility of the isolate Mucor sp.(Mu3) to be a suitable source for oil and PUFA production.

KEYWORDS: Single Cell oil, Isolate, Mucor sp., Fatty Acids, Polyunsaturated Fatty Acids

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INTRODUCTION

The Interest in the applications of the PUFA was increased in all fields, particularly healthy and nutrition applications, making the attention focused towards the availability of alternative sources of permanent and suitable for the production of these compounds. The isolation of oleaginous microorganisms with high efficiency production of (PUFA) led to the development of fermentation technologies as a convenient alternative to traditional sources of agricultural and animal -mediated fungi and microalgae. These prompt the idea of increasing the production level of these acids. This can be made by employing new strategies such technologies mutagenesis, molecular engineering, selection of new microbial strains and determine the optimum conditions for the strains that have more efficient production (Certik and Shimizu, 1999; Ahmed et al., 2006).Unique of the PUFA in turn organizational physiological and

functional cell organelles such as Phase transition, Membrane permeability and the work of the proteins associated with the membrane (Horrobin, 1995),as well as the responsibility for adapting the cell by modifying and modulating expression genes (Sessler, Ntambi, 1998).

PUFA are precursor's compounds to the Prostaglandins synthesis. it play a major role in regulating blood pressure, clotting and stimulate the immune system, as well as organize the work of the heart, kidney, lung, liver, and brain (Restek, 2000). The term Single Cell Oils (SCO) demonstrates the production of oils and lipids from cells microbiology, provided the proportion of oil ≥ 20% of the total dry biomass called Oleaginous microorganisms that have the ability compilation lipid in their plasma membranes compared to Non-oleaginous Microorganisms that can not only assembling lipid ratios less than 10%, based on studies of Ratledge (Ratledge, 1991).

Emerged types belonging to the genera such as Zygorhynchus, Cunninghamella, Rhizopus, Mortierella and Mucor in their high potential building and assembling copious amounts of lipid more than 25 %, that prevails in the form triglyceride(TAG) with rich in their content of PUFA both omega-3 and omega -6 lead to increase interest in them and make these genera at the forefront of Oleaginous microorganisms within SCO at a commercial production with scalable and increasing (Webster and Weber, 2007; Kavadia et al., 2001). In particular essential fatty acids(EFAs) like Linoleic acid (LA), α-Linolenic acid (ALA), ϒ-Linolenic acid (GLA), Dihommo-ϒ-linolenic acid (DHGLA), Arachidonic acid (ARA), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), very long-chain (VLCPUFA) according to (Certik and Shimizu, 1999; Mamatha et al., 2010).

This study aimed to obtain isolates from local and non-pathogenic fungi suitable sources for the production of PUFA substitute for traditional sources of plant, animal, the literature reviews show a lack of studies and previous research relating to this area especially in Iraq, as well as the investment of the agricultural waste and the industrial waste food related costs as alternative sources of cheap nutritious isolation optimal use in the production of the PUFA.

MATERIALS AND METHODS

The Chemicals

All solvents and compounds used in the research were of a high pure level analytical grade. These included glucose, Peptone from Difco(USA), Agar,Yeast extract from Oxide(England), Crystalline phenol (NH4OH) ammonium hydroxide,(NaOH) Sodium hydroxide ,Butylated Hydroxy Toluene(BHT), o-Phenanthroline monohydrate from BDH(England), Glycerol ,Lactic acid ,Cotton blue ,(KH2PO4)Potassium dihydrogen phosphate (K2HPO4)Dipotassium hydrogen phosphate from Fluka(Germny), (K2Cr2O7)Potassium dichromate, sulphate heptahydrate (FeSO4.7H2O) from Supleco(USA), (HCL)Hydrochloric acid, (NaCL)Sodium chloride, Chloroform from Scharlau(Spanish), (CH3OH) Methanol from Hayman(England), n-Hexane from GCC(England), Chloramphenicol from Globalpharma(UAE), Boron trifluorid dehydrat (BF3.2H2O), Dimethyl sulfoxide(DMSO), Heptadecanoic acid(17:0) from Sigma Aldrich(USA), Potassium nitrate(KNO3), Ammonium chloride (NH4CL), Magnisum sulphate pentahydrate (MgSO4.5H2O) form Merek(Germany).

The Cultures and Media

All solid and liquid media were prepared with the sterilized and distilled water and sterilized in autoclave at 121o C and under pressure of 15 lbs / Inch ² for 15-20 minutes, 250 mg / liter of Chloramphenicol were added before the sterilization to prevent any bacterial contamination.

Sources of the Fungal Isolates

Soil samples were collected according to Tornisielo et al. (Tornisielo et al., 2007), The samples were rice bran, dairy, meat, potato tubers, all samples were obtained from local markets according to Wadi (Wadi, 2009). All the samples were collected during March and April, 2012.

Isolation, Purification and Diagnose of Isolates

The procedures were followed according to Ahmed et al. (Certik and Shimizu, 1999), the isolation of molds from soils, rice bran and milk collected samples were performed using a series decimal dilutions by spreading on the PDA (Hemidia Company), while the rotting potatoes and meat were performed by direct method (Devi et al., 2006). Two duplicate of each sample were made, two Petri dishes without inoculation as a control. All the dishes were incubated at 30 oC for a period of 3-5 days with daily growth monitoring.

Isolates were purified according to Becuchat, (Beuchat, 1992) and Kavadia et al. (Kavadia et al., 2001) by Sub-culturing several times that inoculation by contact of needle sector in the center of PDA petri dish media and incubated pure isolates in sterile tubes container with slant PDA at 4 -7co in duplicate. The isolates were renewed periodically every 15-30 days for the purpose of diagnosis and production.

Diagnosed pure isolates were conducted in two phases, the first one is on the basis of phenotypic characteristics of the colonies developing, and the second one is on microscopic. This part of the work was done in the (Central laboratory fungi - College of Science and Laboratory fungi in prevention department - Faculty of Agriculture - University of Basra) based on the keys Category scientific mentioned by Becuchat, (Beuchat, 1992) and Pitt and Hocking (Pitt and Hocking, 1997).

Preparation of the Commenter Spores

The suspended of inoculums was attended according to the method of Kamlangdee and Fan (Kamlangdee and Fan, 2003), Using Glucose -Yeast extract - Peptone Agar (GYP) according to a Jeennor et al. (Jeennor et al., 2006). The spores were counted by the Haemocytometer under the compound optical microscope, the concentration of the inoculums was 10 6 -107 spore\ml.

Primary Screening

The screening was conducted according to Kavadia et al(Kavadia et al., 2001) and Ahmed et al. (Ahmed et al., 2006) and submerged fermentations (Smf) system with some minor modifications which necessitated that need for the purpose of screening isolates which is capable to produce oily content by 20% ≥ of total dry weight were used. Using of the Glucose-Peptone - Yeast extract fortified modified broth (GPYB) was reported by Liu et al. (Liu et al., 2010) with some modifications in concentrations, the pH was adjusted to be between 6-7 and then completed to one liter with distilled water and distributed in Erlenmeyer flasks of 250 ml, each flask contains 50 ml of the base broth and closure by cotton plugs covered with aluminum foil and cooled after sterilization to room temperature. Then, inoculated inside sterilization cabin with 1 ml of the suspension spores for each isolation which contains between 106 - 107 spore/ml and three replicates for each isolated. Finally, the samples placed in the shaking incubator of 200 stroke / minute at 28 ± 1 oC for 6-7 days.

Estimating of the Weight of Dry Biomass

The bulk growth was separated by vacuum filtration with Buchner Funnel through filter paper Whatman No.1,

thoroughly washed three times with 50 ml of distilled water for each duplicate, each isolate individually was freeze dryied (Lyophilization) at - 40 oC then weighed dry biomass gravimetrically by delicate balance (g / l) according to Kavadia et al. (Kavadia et al., 2001) and Aminah et al. (Aminah et al., 2006).

Lipid Extraction

Method of Folch et al. (Folch et al., 1957) was adopted as basis for lipid extraction from dry biomass for three replicates each individual isolate. The lipid amount in gram was calculated using the equation: The amount of lipid extracted = weight of the tube with extracted lipid - the weight of an empty tube. The percentage of the total content of single cell oil was calculated using the bellow equation:

% Oil content = weight of the extracted oil (g) \ weight of the original sample (g) × 100.

Bioassay Detection of Fungal Oil Safety

By utilizing brine shrimp Artemia salina leach bioassay as a vital test adopted to detect any mycotoxin activity of extracts fungal isolates according to Al-Hazmi (Al-Hazmi, 2010) and calculate the percentage of mortality and through identifiy the effectiveness toxic of lipid extract for each isolation at concentrations of 3000 ppm and 5000 ppm.

Secondary Screening of Isolates Producers of PUFA Qualitatively and Quantitatively

Method of Kang and Wang (]Kang and Wang, 2005) was adopted as a basis for the preparation of fatty acid methyl esters with some additions which found that they raise the quality and improve the analysis based on the Liu et al. (Liu et al., 2010) mediated catalyst esterification of 14% Methanolic Boron Trifluoride (BF3-MeOH) and hexane in a tube esterification. The upper layer that contained fatty acid methyl esters was drawn for injection.

The analyses of SCO lipid, determination of the PUFA profile and the estimation of the quantity of each acid were done in the Central GC-MS laboratory - College of Agriculture / University of Basra using Gas chromatography-Mass spectrometry (GC-MS), type QP 2010 Ultra, Shimadzu-Japan, the separation column type is DB-1 ms with a length of 30 m and a diameter of 0.25 µm, the wall thickness is 0.25 mm, the carrier gas was the helium. The identification of the types of fatty acids, and especially PUFA was done through confirmation compared with the Retention Times (RT) in the separation column and the mass spectrum parts broken mass spectrometric fragmentation with compounds (fatty acids) original Authentic adopted in the spectral data stored in the electronic library of the computer linked to a GC / MS according to Ahmed et al. (Ahmed et al., 2006), it has been relying on the program Library NIST 0.8 LIB, according to Devi et al. (Devi et al., 2006) and Mamatha et al. (Mamatha et al., 2010) in using of the relative percentage of area peaks and the height intensity of fatty acids among the isolates to determine the most prolific and ratio of PUFA during the secondary screening.

The internal standard heptadecanoic acid (17:0) method estimates the amounts of PUFA in the lipid of optimum isolate through selected carbon and nitrogen sources according to Kamlangdee and Fan (Kamlangdee and Fan, 2003) and Athalye (Athalye, 2008), the quantity is estimated in mg / L.

The Substrates Preparation

The carbon and nitrogen sources were obtained from agricultural wastes, animal and industrial waste food according to Issa (Issa, 2013).

The Walkley-Black procedure that depicted by Nelson and Sommers (Nelson and Sommers, 1982) was followed to estimate the proportion of the total percentage of the organic carbon. The method which adopted Sime-MicroKjeldahl was described by Pearson (Pearson, 1971), it was used to calculate the ratio of the total percentage of the nitrogen.

The Optimum Carbon and Nitrogen Sources

Rice bran, wheat bran, potato peel, whey and date seed powder were adopted as carbon source to determine the organic carbon source and total nitrogen. These sources were added at 7.5%gm/ml to primary screening media to replace glucose, the other components and growth conditions for media weren't change. The spleen powder, chicken feather and alfa alfa were utilized as an organic nitrogen source, the yeast extract and peptone were reused but in new concentrations were replaced the organic and inorganic nitrogen sources in the primary screening media were adopted with one of these sources at 0.1% gm/ml with fixed the optimal carbon source as mentioned above.

Statistical Analysis

The results were analyzed statistically using Analysis of Variance (ANOVA). The difference averages were tested using less significant difference (LSD) at the level of probability of 1% using the ready statistical program (Statistical Package for Social Science (SPSS 15.0; SPSS Inc.)) according to the study of Khongto et al. (Khongto et al., 2010).

RESULTS AND DISCUSSIONS

Isolation, Purification and Identification of Fungal Sources

In this study eight pure isolates were obtained and identified morphologically and microscopically by specialist and belong to the genus Mucor.

Primary (Quantitative) Screening

Table 1 showed the results of the fungal isolates capacity to produce lipids at rates exceeding 20% of dry biomass according to SCO principle, according to Ratledge (Ratledge, 1997) and Ratledge and Wynn (Ratledge and Wynn, 2002).The primary screening trials showed seven isolates were capable to produce SCO at a rate exceeding 20%, the isolate *Mucor* sp. (Mu3) was superior to other in producing the highest dry biomass and oil content. The isolate Mu1 was failed to be an oil producing isolate. The obtained results was better than that of Ahmed *et al*. (Ahmed *et al*., 2008), who used the isolates *Mucor hiemalis* MTCC (1277, 157) and Li *et al*. (Li *et al*., 2008), on the isolate *Mucor recurvus* for the producing of the SCO using sucrose and starch instead of glucose.

Table 1: Primary Screening of Fungal Isolates for Oil Production at a Rate of 20% of Dry Biomass (Results are the Average of Duplicates for Each Isolate)

	Fungal Isolate	Isolate Source	Dry Biomass g/l	Total Lipid g/l	Lipid % w/w
1	*Mucor* sp. (Mu1)	Dry soil	6.332	1.1341	16.32
2	*Mucor* sp. (Mu2)	Garden soil	8.332	2.4208	29.05
3	*Mucor* sp. (Mu3)†	Oily soil	10.963	5.4739	49.93
4	*Mucor* sp. (Mu4)	Rice bran	10.261	4.6929	45.73
5	*Mucor* sp. (Mu5)	Local potato	8.996	2.7971	31.09
6	*Mucor* sp. (Mu6)	Local cream	7.425	1.7219	23.19
7	*Mucor* sp. (Mu7)	Buffalo yogurt	6.634	1.5098	22.75
8	*Mucor* sp. (Mu8)	Rancid sheep fat	7.592	1.8209	23.98

Biological Examination of the Extracted Oil from Oily Isolates

According to Abdel-Malek *et al* (Abdel-Malek *et al, 1993*), the oily fungal isolates cause high death rate to *Artemia salina* and was within low or non toxic range. This may allow the isolated compounds or metabolites to be utilized in food systems. Table 2 showed the safe use of SCO from the seven *Mucor* species. This result was agreed with Webster and Weber(Webster and Weber, 2007) investigations that showed incapacity of most of species to produce toxin.

Table 2: The Safety Biological Test of Oily Isolates

	Oil Extract Concentration / Fungal isolates	3000 ppm		5000 ppm		Mortality Rate† %	Toxic Degree
		Mortality Percentage of Larvae after 48 & 24h					
		24 h.	48 h.	24 h.	48 h.		
A	control tube(1) without oily extract	0	0	0	5	1.25	———
B	control tube(2) without oily extract	0	0	0	3	0.75	———
1	*Mucor* sp. (Mu2)	0	0	0	10	2.5	Non-toxic
2	*Mucor* sp. (Mu3)	0	0	0	0	0	Non-toxic
3	*Mucor* sp. (Mu4)	0	5	10	20	8.75	Non-toxic
4	*Mucor* sp. (Mu5)	0	0	0	10	2.5	Non-toxic
5	*Mucor* sp. (Mu6)	0	5	5	15	6.25	Non-toxic
6	*Mucor* sp. (Mu7)	0	0	5	10	3.75	Non-toxic
7	*Mucor* sp. (Mu8)	5	5	10	15	8.75	Non-toxic

More than75%=High-toxic, 50-75%=Toxic, 50-25%=Weak-toxic, less than 25%=Non-toxic.

Quantitative and Qualitative Analysis of Fatty Acids

Table 3 showed the fatty acids profiles produced from secondary screening isolates as determined using GC-mass chromatography. The obtained results showed that the isolate *Mucor* sp.(Mu3) was the best one for quantitative production of PUFAs, as well as, qualitatively for GLA,EPA,DPA and DHA and fair amounts of both LA and ALA. The obtained results were superior for both LA and GLA to that of Ahmed *et al*.(Ahmed *et al*.,2006) for *Mucor* sp. RRL001 that failed to produce other fatty acids. Furthermore, both LA and ALA contents were higher than Liu et al. (Liu et al., 2010) investigations on *Thamnidium ctenidum* that were incapable to produce other fatty acids obtained in present study, thus *Mucor* sp.(Mu3) was selected as the best isolate.

Table 3: Ratio and Fatty Acids Profiles Produced From Local Fungal Isolates as Assayed by GC-mass Chromatography

Fungal Isolate	Source	% for Each Fatty Acid of Total Lipid Content from Oily Isolates					
		LA	GLA	ALA	EPA	DPA	DHA
Mucor sp.Mu2	Garden soil	10.97	6.87	0.63	—	—	—
Mucor sp. Mu3	Oily soil	11.78	10.95	3.02	4.6	0.39 (GO-3)	1.47
Mucor sp. Mu4	Rice bran	18.44	0.34	4.93	0.84	—	—
Mucor sp. Mu5	Local potato	12.24	2.09	—	—	0.24 (GO-3)	—
Mucor sp. Mu6	Local cream	16.37	8.55	0.31	—	—	—
Mucor sp. Mu7	Buffalo yogurt	13.23	5.63	—	—	—	—
Mucor sp. Mu8	Rancid sheep fat	20.35	—	4.17	—	—	—

*LA=Linoleic acid, GLA=gamma-Linolenic acid, ALA=α-Linolenic acid, EPA=Eicosapentaenoic acid, DPA=Docosapentaenoic, DHA=Docosahexaenoic acid.

Selection of Substituted Substrates as Carbon and Nitrogen Sources

The raw materials as carbon and nitrogen sources to substitute glucose in primary screening media were selected on carbon: nitrogen ratio that considered as critical and limited factors in SCO production as concluded by Saenge *et al*. (Saenge *et al*., 2010) when carbon in glycerol and nitrogen in ammonium sulfate were assayed to obtain best oil ratio from *Rhodotorula glutinis.*

Table 4: Carbon and Nitrogen Content in Substituted Raw Materials (Results Represent Average of Triplicate

Raw materials	TOC%	Total(N)%	C/N	Type
Rice bran	55	1.89	29.11	Carbonic source
Wheat bran	50	2.18	22.93	Carbonic source
Potato peel powder	44.5	1.56	28.52	Carbonic source
Whey	45.4 ~45.5	1.96	23.21	Carbonic source
Date seeds powder	52.5	1.17	44.87	Carbonic source
Spleen powder	41.6	11.8	3.52	Nitrogen source
Chicken feathers powder	47.3 ~ 47	13.5	3.48	Nitrogen source
Yeast extract	31.7 ~ 32	10.5	3.04	Nitrogen source
Peptone	39.09 ~39	13.6	2.86	Nitrogen source
Alfa alfa powder	46.23 ~ 46	4.6	10	Nitrogen source

The Carbon Source

The primary screening media was substituted for different raw materials as carbon source, that value 7.5% w:v and equal to 3% glucose was used in primary screening. This was adapted by Zhu (Zhu, 2002) when 20% glucose was substituted for 5% rice bran for producing SCO, ARA and EPA from *Pythium irregular* in immersed liquid culture. Table 5 showed biomass and total oil quantity produced by dried biomass from substituted carbon source from *Mucor* sp.(Mu3).

Table 5: The Effect of Carbon Source on Producing Dried Biomass and Total Oil from Mucor sp.(Mu3) (The Results of Triplicates ± the Standard Deviation)

Carbon Source	Dried Biomass g/L of Media	Total Lipid g/L of Media	%Total Lipid of Dried Biomass
*Rice bran	25.77 ± 0.98	8.36 ± 0.1	32.4 ± 1.16
Wheat bran	22.1 ±0.66	5.260.34 ±	23.82 ± 1.44
Potato peel powder	19.97 ± 0.3	5.290.37 ±	27.01 ± 2.9
Whey	15.28 ± 0.32	3.27 ±0.33	21.4 ± 1.79
Date seeds powder	7.59 ± 0.53	1.33 ± 0.06	17.6 ± 1.73
L.S.D	1.58	0.71	5.08

*The results showed rice bran was superior significantly ($p< 0.01$) to other carbon sources as related to dried biomass, total lipid content and production rate.

Table 6 showed fatty acids profile of produced lipid(oil) by *Mucor* sp.(Mu3) from different carbon resources, rice bran was superior to other carbon sources as produced different and versatile fatty acids, particularly both ω-3 and ω-6 as in shown figure 1 that are important nutritionally, namely LA, ALA, GLA, EPA, DPA and DHA as in figure1) the GC/Mass analysis, but the ARA couldn't be produced by all these resources.

It can be concluded that *Mucor* sp.(Mu3) isolate utilized rice bran is better than the other sources and this due to N:C ratio and it was the best among other sources and fatty acids profile particularly ARA and LA according to Jang *et al*. (Jang *et al*., 2000). On the other hand, Zhu (Zhu, 2002) explained the unique composition of rice bran for nutrient contents that can be utilized easily by mold when *Pythium irregulare* was utilized for producing SCO, ARA and EPA. This was in

agreement with Jang *et al*. (Jang *et al*., 2000)as rice bran was superior substrate to other resources as far as SCO and PUFA contents production by *Mortierella alpina* is concerned.

Table 6: The Effect of Carbon Sources on PUFA Production by Mucor sp. (Mu3)

Carbon Source	PUFA Content(mg/L) of Media					
	LA	GLA	ALA	EPA	DPA(ɷ-3)	DHA
	w/v	w/v	w/v	w/v	w/v	w/v
Rice bran	636.75	89.45	126.78	185.34	61.2	128.42
Wheat bran	543.33	46.6	106.67	76.74	24.8	44.63
Potato peel powder	213.31	32.31	104.85	25.57	32.6	—
Whey	186.56	37.94	26.3	14.26	—	—
Date seeds powder	No results due to the total lipid was less than 20% of dried biomass					

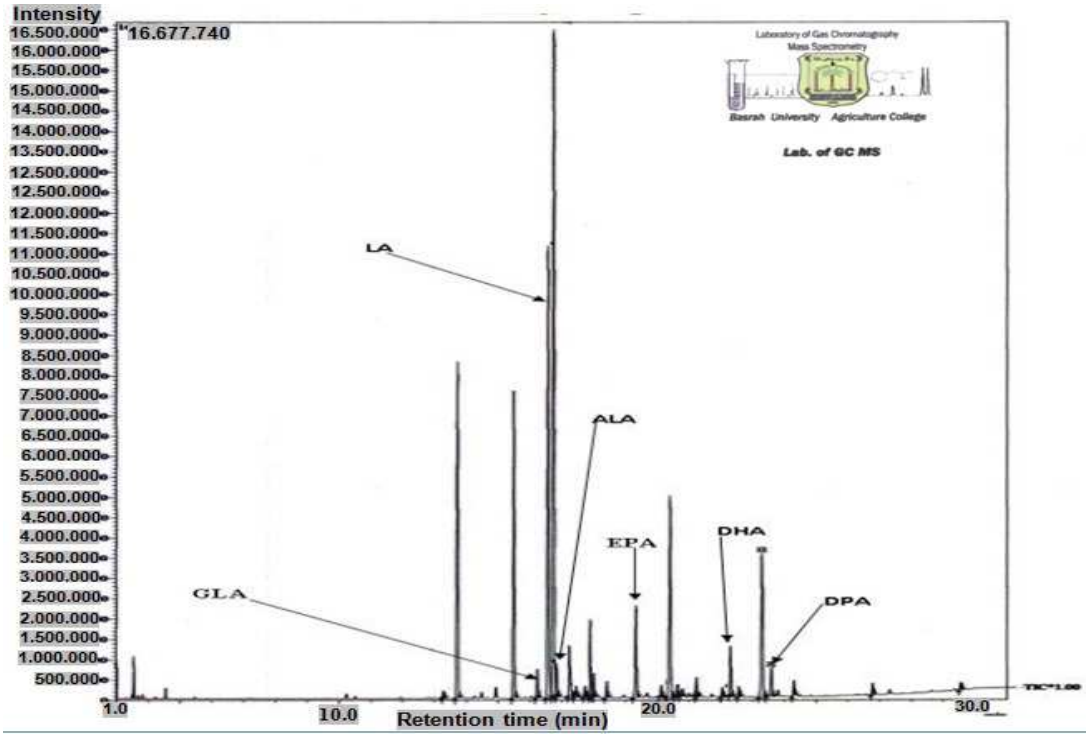

Figure 1: GC-Mass Analysis and Peaks of Fatty Acid methyl Esters from Extracted Lipid Produced by Mucor sp.(Mu3)Utilized Rice Bran

The Nitrogen Sources

Table 7 showed the obtained results from *Mucor* sp.(Mu3) when different organic raw materials were utilized as supported nitrogen sources. It was appeared that yeast extract was superior significantly ($p<0.01$) in producing dried biomass and total lipid content. The statistical analysis didn't show any significant differences among the used raw material, though; chicken feather was superior on other raw materials as showed in higher oil produced. This may be due to the isolate(Mu3) utilized all nitrogen sources within different rates and this related to the chemical nature of components and solubility in the media that simplified utilization of such materials.

Table 7: The Effect of Nitrogen Sources in Producing Dried Biomass and oil by Local Isolate Mucor sp.(Mu3) (The Results of Triplicates ± the Standard Deviation)

Nitrogen Source	Dried Biomass g/L of Media	Total Lipid g/L of Media	Dried Biomass g/L of Media
Spleen powder	14.14 ±0.42	4.13 ±0.19	29.25 ±2.08
Chicken feathers powder	11.82 ±0.51	4.1 ± 0.37	43.7 ± 2.88
Yeast extract	17.83 ±0.28	5.65 ±0.68	31.84 ±3.14
Peptone	13.7 ±0.51	4.29 ±0.04	31.34 ±1.03
Alfa alfa powder	9.22 ±0.85	2.47 ±0.04	26.8 ± 0.19
L.S.D	1.02	0.93	5.62

Based on PUFA content in oil produced from the isolate(Mu3) for each nitrogen sources, Table 8 showed that chicken feather was the best source for producing all PUFA that produced from optimum carbon source, while, both DPA and DHA failed to produce or with impalpable amount from other sources. Quantitatively, for these fatty acids, chicken feather was the best nitrogen source for supporting rice bran that was superior to other nitrogen sources for producing PUFA types, figure 2 show oil GC/Mass analysis.

Table 8: The Effect of Nitrogen Sources on PUFA Production by Mucor sp. (Mu3)

Nitrogen Source	PUFA Content(mg/L) of Media					
	LA	GLA	ALA	EPA	DPA(GϽ-3)	DHA
	w/v	w/v	w/v	w/v	w/v	w/v
Spleen powder	361.42	39.81	54.33	60.63	—	—
Chicken feathers powder	596.74	93.5	142.63	183.2	49.45	119.27
Yeast extract	422.63	41.82	52.72	19.35	—	—
Peptone	383.81	44.67	54.72	17.86	—	—
Alfa alfa powder	374.62	62.75	67.47	72.34	2.57	6.25

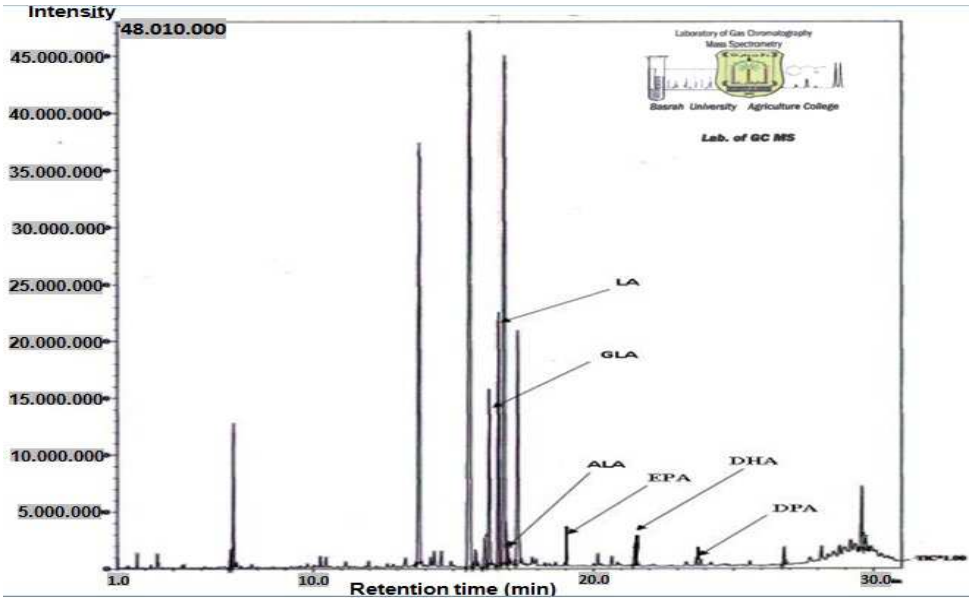

Figure 2: GC-Mass Analysis and Peaks of Fatty acid Methyl Esters from Extracted Lipid Produced by Mucor sp. (Mu3) Utilized Chicken Feather Powder

The obtained results were agreed with Dyal *et al*. (Dyal *et al*., 2005) results as both yeast extract and peptone

promotes production of biomass and total oil as nitrogen sources supported *Mortierella ramanniana* to produce low GLA and other omega-3 fatty acids but with high level of LA and saturated fatty acids. This was also in agreement with De Silva *et al*. (De Silva *et al*., 2006) in low production of DHA from *Crypthecodinium cohnii* with yeast extract as nitrogen source and n-dodecane as carbon source, increasing yeast concentration ceased DHA production. The obtained results was agreed with Li *et al*. (Li *et al*., 20008) as organic and inorganic nitrogen sources and molasses as carbon source employed with *Mucor recurvus* and showed higher biomass formed with low lipid and PUFA production. It was concluded that organic nitrogen sources were better than inorganic nitrogen sources.

It can be concluded that chicken feather powder was the best supporting nitrogen source for producing types of PUFA.

CONCLUSIONS

The recent study showed that it could be screened the oleaginous fungi by submerged batch cultures to find the optimum strain resembled with *Mucor sp*.(Mu3) which proved its non toxic fungus by bioassay test and as suitable organism can be utilize variety types of cheap raw materials as carbons and nitrogen's sources that can replaced other than typically media that lead to reduce cost for producing SCO as an alternative energy source as biodiesel with balanced PUFA content that important nutritionally for human health especially GLA,EPA and DHA.

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